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FOREWORD

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Nicholas Ting, Ph.D. 4/10/00

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Annual Report for Award Number DAMD17-97-1-7205

Principal Investigator: Nicholas S.Y. Ting, Ph.D (Post-Doctoral Fellow)
Supervising Investigator: Wen-Hwa Lee, Ph.D

Report Title: Regulation of BRCA1 Function by Phosphorylation.

Introduction:

Mutations to the *BRCA1* gene are responsible for nearly 50% of inherited cases of breast cancer. However, the precise mechanism by which BRCA1 contributes to the progression of tumour development remains to be elucidated. Recent evidence have suggested that BRCA1 is important for cellular DNA damage repair and/or DNA damage response pathway [reviewed in 1, 2]. First, mechanistic studies have shown that BRCA1 associates with the DNA repair protein triplex, Mre11/Rad50/NBS1 to form distinct nuclear foci that correlates to sites of DNA damage in cells treated with genotoxic agents [3]. Second, overexpression of BRCA1 induces the expression of GADD45 and p21, two DNA-damage responsive genes [4,5]. Based on these reports, it is has been proposed that absence of functional BRCA1 leads to aberrant repair of DNA, which in turn, can lead to genomic instability and ultimately, tumourigenesis.

Previous work in our laboratory has shown that BRCA1 is a 220 kDa nuclear protein that becomes hyperphosphorylated in a cell-cycle dependent manner [6]. BRCA1 also becomes hyperphosphorylated when cells are treated with genotoxic agents [4,7]. We also noted that a BRCA1 associated protein, called CtIP (for C-terminal interacting protein), is also phosphorylated upon exposure of cells to ionizing radiation (IR). CtIP was originally isolated by its association with the adenovirus E1A co-repressor protein CtBP [4]. CtIP binds to the C-terminal region of BRCA1, and this association is disrupted upon IR *in vivo* [4]. Co-expression of BRCA1 with CtIP and co-repressor CtBP, is able to repress BRCA1-mediated transcriptional activity from the p21 promoter (4). Therefore, we were interested in identifying the protein kinase that is responsible for this DNA damage induced phosphorylation of CtIP and BRCA1, and study how this event contributes to the function of BRCA1 in the DNA damage repair and response pathway.

Experimental Strategy and Results:

To this end we initially tested the DNA-damage induced phosphorylation status of CtIP and BRCA1, in cell lines deficient for two protein kinases involved in DNA damage signaling: DNA-dependent protein kinase (DNA-PK) and ATM (for mutated in Ataxia Telangiectasia) [reviewed in 8]. In DNA-PK deficient cells, the IR induced phosphorylation of BRCA1 and CtIP was observed. On the other hand, the IR induced phosphorylation of CtIP was absent, while IR induced phosphorylation of BRCA1 was slightly compromised in ATM deficient cells. Because the reduction of IR induced phosphorylation of BRCA1 was not as prominent compared to CtIP in the ATM deficient cells, we concentrated our studies on CtIP. Transfection of wild type ATM into ATM

deficient cells restored IR-induced phosphorylation of CtIP, suggesting that CtIP is a target for ATM kinase following DNA damage.

Ataxia Telangiectasia is a rare autosomal recessive genetic disorder characterized by immune system deficiencies, growth retardation, neuronal degeneration, and a 100 fold increase in the incidence of some cancers such as leukemia and lymphoma [reviewed in 9]. The gene product encodes for a 220 kDa serine/threonine protein kinase that is required for p53 activation following DNA damage [8]. Inspection of the amino acid sequence of CtIP revealed that it has several ATM kinase consensus phosphorylation sites, but only two sites are conserved in mouse and human, Ser 664 and Ser 745. We replaced these two serine residues to alanine, and expressed recombinant wild type CtIP or mutant CtIP (S664/745A) and asked whether they are substrates of ATM. Immunoprecipitated ATM was able to phosphorylate CtIP(wt), but not CttP(S664/745A) *in vitro*. We then performed two-dimensional phosphopeptide maps to show that Ser 664 and Ser 745 on CtIP are phosphorylated *in vivo* following IR.

The next set of experiments were performed to determine the consequence of IR induced ATM-dependent phosphorylation of CtIP. Immunoprecipitation studies indicated that ATM exists in the previously determined CtIP-BRCA1 complex *in vivo* [4]. Following DNA damage, CtIP dissociates from BRCA1; however, in ATM deficient cells, the association between BRCA1 and CtIP persisted after IR. We then transfected flag-tagged CtIP(wt) or CtIP(S664/745A) into ATM proficient cells and performed immunoprecipitation studies with anti-flag antibodies. Following IR, the CtIP(wt)/BRCA1 complex was disrupted, but not the CtIP(S664/745A)/BRCA1 complex, suggesting that ATM dependent phosphorylation of Ser 664 and Ser 745 on CtIP is required for dissociation of CtIP from BRCA1 following DNA damage.

We have shown that BRCA1 can promote transcriptional activity from the p21 promoter [4]. It has been recently shown that BRCA1 induced the expression of *GADD45*, and this induction requires the intron 3 region of *GADD45* [5]. To further investigate the significance of IR-induced ATM-dependent phosphorylation of CtIP, we assayed transcriptional activity using a luciferase reporter construct containing the intron 3 sequence of *GADD45* (pI3). As expected, expression of BRCA1 alone induced pI3 reporter activity by about 5 fold in human osteosarcoma U20S cells (ATM proficient). Co-expression of BRCA1 with CtIP/CtBP led to repression of pI3 reporter activity, which was relieved following exposure of cells to IR. This derepression was not seen with cells co-expressing BRCA1 with the mutant CtIP(S664/745A) and CtBP, suggesting that phosphorylation on these serine sites is critical for relief of repression. Consistently, the derepression of transcriptional activity from the pI3 reporter was not observed in ATM deficient cells following IR treatment. Taken together, these data suggest that IR induced phosphorylation of CtIP on Ser 664 and Ser 745 by ATM is required to dissociate CtIP/CtBP from BRCA1, enabling BRCA1 to participate in the expression of *GADD45*.

Conclusions:

These results are reported in a manuscript entitled, "ATM-dependent phosphorylation of CtIP mediates induction of GADD45 expression by BRCA1 upon γ-irradiation," which has been resubmitted for a second round of review in *Nature* [10]. We are confident that it will be accepted for publication, as we have completed all of the suggested experiments from the first set of reviews. During the preparation of this manuscript, the Elledge group has demonstrated that Ser 1423 and Ser 1524 on BRCA1 is phosphorylated by ATM in response to IR [11]. Mutation of these serines to alanine, resulted in aberrant BRCA1 mediated cellular response to DNA damage [11]. Combined with our data, a link between ATM and BRCA1 has been established, which may explain the increased risk of breast cancer in certain Ataxia Telangiectasia heterozygotes [12]. It is likely that in one DNA damage response pathway ATM transduces damage signal by phosphorylating CtIP and BRCA1 (Figure 1). Phosphorylation of CtIP by ATM dissociates BRCA1 from the CtIP/CtBP repressor complex, subsequently enabling BRCA1 to partake in the induction of p21 and GADD45 (Figure 1). In the absence of functional ATM, the activity of BRCA1 may become disregulated leading, in turn, to a defect in the cellular response to DNA damage, which may lead genomic instability and tumourigenesis.

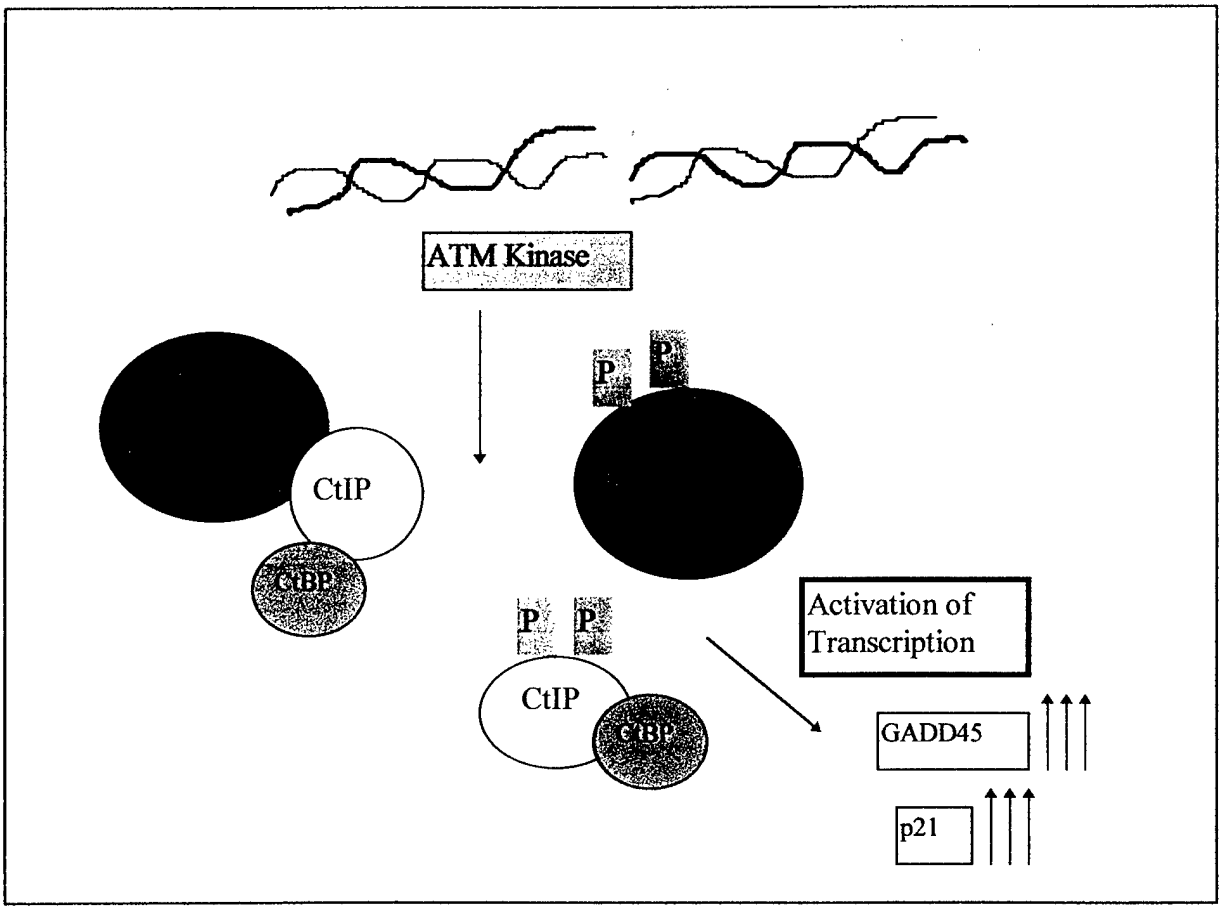

Figure 1: Model for BRCA1 function following IR regulated by ATM.

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